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ORGANIC MATRIX COMPOSITION OF MODERN AND 8.7K BP *MYA TRUNCATA* (MOLLUSCA: BIVALVIA) FROM ARCTIC CANADA

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ABSTRACT

Amino acid and amino sugar compositions of the organic matrices of modern and subfossil *Mya truncata* shells from Arctic Canada were determined by high-performance liquid chromatography analyses. Comparison of compositions of modern and subfossil hydrolyzed soluble and insoluble matrix residues reveals that modern and 8.7k BP samples show statistically significant differences resulting from post-mortem alteration. These findings suggest the potential geochronological usefulness of matrix residues from *M. truncata* subfossil shells. Nevertheless, post-mortem hydrolysis of the insoluble matrix likely contributes to the composition of the soluble fraction collected from subfossil shells. This process hampers defining the true composition of the subfossil soluble matrix and inferring soluble matrix diagenesis.

Introduction

Mollusk shells contain a small amount (≤5% by weight) of organic matter called an organic matrix. This matrix is divided into soluble or insoluble components depending on its behavior in decalcifying media and acids. The matrix is both an agent and a product of biomineralization processes (Lowenstam and Weiner, 1989).

One or two amino acids, usually glycine or glycine and alanine, are the principal monomeric components of mollusk insoluble matrices. These and other amino acids function as monomeric constituents of hydrophobic proteins (Meenakshi et al., 1971; Grégoire, 1972). Amino sugars, such as galactosamine and particularly glucosamine, are also important parts of the insoluble matrix of many species. Glucosamine is the monomeric constituent of chitin, which is located between protein sheets (Goffinet and Jeuniaux, 1969; Weiner and Traub, 1980). Other matrix carbohydrate monomers include the monosaccharides altrose, fucose, galactose, glucose, mannose, and xylose, which have been identified in the form of silylated derivatives. The role of some of these and other carbohydrates in the matrix may be as carbohydrate constituents of glycoproteins (Tevesz et al., 1992; 1994).

Sulfated glycoproteins comprise a major portion of the soluble matrix (Simkiss, 1965; Crenshaw, 1972). These proteins are rich in aspartic acid and can bind calcium ions. The two fractions of the organic matrix come from two different sources within the shell. The insoluble matrix is mainly located between crystals of calcite or aragonite, while the soluble matrix has an important intracrystalline component.

The geological use of information from organic matrices began when Ableson (1954) reported the occurrence of amino acids in fossils. Subsequent investigations have focused on the source, distribution, composition, and diagenesis of matrix constituents. An important geological use of information from insoluble matrix has been as a source of absolute ages and as biostratigraphic correlation tools. This work is based on time-related changes in ratios of matrix amino acids and also the extent to which certain amino acids have undergone racemization (Wehmiller, 1990; 1993).

Because the soluble component of the organic matrix has an intracrystalline component, some workers consider it to be more resistant to diagenetic change than the more "exposed" insoluble matrix. Thus, it has not been used as much for chronological work as has insoluble matrix but has proven useful as a source of information for taxonomic and phylogenetic studies (Muyzer et al., 1988; Lowenstam and Weiner, 1989; Robbins et al., 1993).

This paper describes and compares for the first time amino acid and amino sugar compositions of the organic matrices of modern and subfossil *Mya truncata* Linnaeus, 1758 (Mollusca: Bivalvia) from Arctic Canada. New data are presented on the soluble fraction and comparisons are made which involve existing insoluble fraction data. In order to facilitate comparisons, we have focused our attention on the most abundant and easily detected amino acids and amino sugars. We describe differences resulting from post-mortem alteration of soluble and insoluble matrix fractions of modern and subfossil shells and comment on the potential geological usefulness of this information.

Mya truncata is an abundant modern marine bivalve and is also common and widespread in Pleistocene and Holocene marine deposits throughout northeastern North America (Aitken, 1987; Aitken and Risk, 1988). The modern specimens for this study were collected alive within a few meters of the subfossil shells, which obviates the possibility of compositional differences between modern and subfossil samples being caused by the sample sets being drawn from geographically distant populations. It is assumed that the 8700 year interval separating the modern and subfossil populations was too brief to permit significant evolutionary changes to occur in the proportions of monomeric constituents of the organic matrix. Thus, compositional differences between subfossil and modern samples likely reflect in situ changes occurring to the matrix within the measured time interval. Because the subfossil specimens collected for this study were from localities in high latitudes, post-mortem matrix alteration was likely minimized because of the low temperatures to which the shells were exposed (Risk, 1991).

Materials and Methods

Well-preserved subfossil shells of *Mya truncata* were collected from raised beaches at Pangnirtung Fiord, Baffin Island, Northwest Territories, Canada (66° 08' N, 65° 43' W). The shells were exposed in a gravel pit. The preservation state of the shells and the geology of the outcrops are described in Aitken (1987) and Aitken and Risk (1988). The C^{14} age of these outcrops is 8700±330 yr BP (Waterloo dating no. 1200). The live modern specimens were collected from adjacent intertidal areas. Shell collections used in this study are archived in the Holocene section of the collections of the Department of Geology, McMaster University.

Thirty-five individuals each of sliced subfossil and modern *Mya truncata* shell pieces were prepared according to procedures described in Tevesz et al. (1992) in order to obtain four matrix samples (modern and subfossil soluble; modern and subfossil insoluble). In addition, shell material was dialyzed in Spectrapor membrane tubing (m.w cutoff: 12,000-14,000; cyl. dia.: 28.6 mm) against approximately 2 liters of a solution consisting of 200 g of EDTA di-sodium salt dissolved in distilled water. The resulting solution was adjusted to neutral pH and then dialyzed against 2.5 liters of distilled water for a period of one week. The contents of

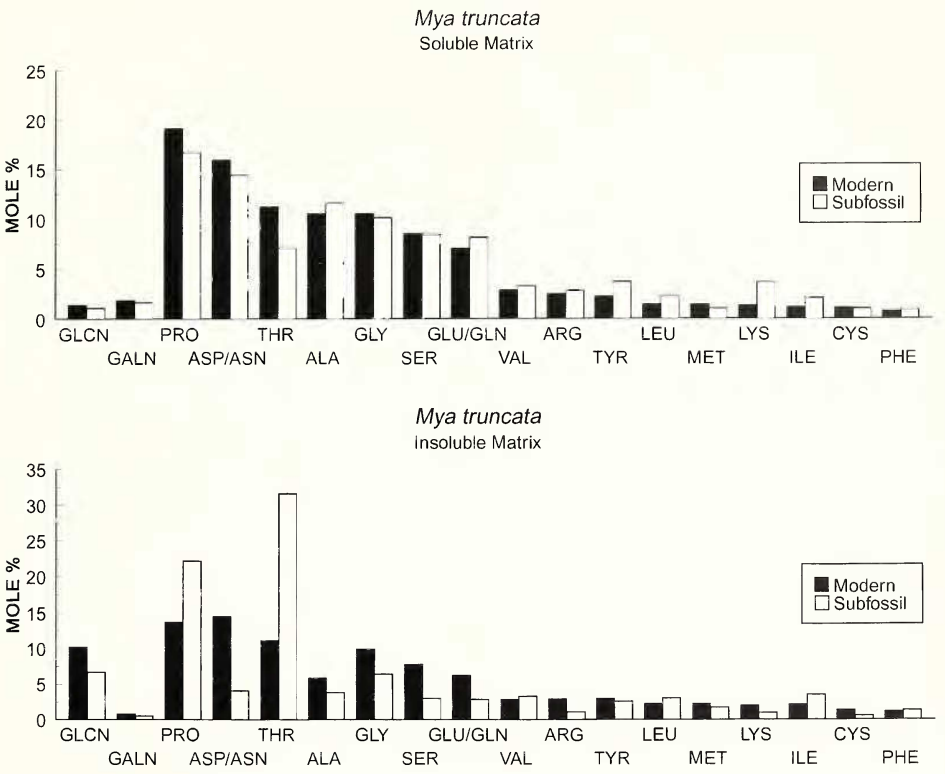

Figure 1. Composition of hydrolyzed matrix residues from *Mya truncata* shells.

the tubing were centrifuged in 50 ml centrifuge tubes. The insoluble matrix fraction appeared as a pellet at the bottom of the centrifuge tube. The supernatant containing the soluble fraction was collected by pipeting and introduced into fresh tubing and dialyzed against three changes of approximately 2.5 liters of distilled water. The contents of the tubing were then placed in flasks and dehydrated under conditions of high vacuum.

Preparation for and analysis by high-performance liquid chromatography (HPLC) of *M. truncata* matrix were done as follows: Matrix samples were dissolved in 2 ml of distilled water, mixed, and then 10 µl of each sample were hydrolyzed in vacuo with gaseous 6N HCl at 105° C for 24 hr using a Picotag work station (Millipore Corporation, Waters Chromatography Division, Bedford, MA). Hydrolysates were derivatized with phenylisothiocyanate (Cohen et al., 1984). The derivatized amino residues were separated by reverse phase HPLC using a 15 cm Picotag Amino Acid Column. Separation was achieved using a linear gradient shifting between polar eluent and nonpolar eluent in 14.5 min. at 42° C. Conditions for separation are described in Table 1. Elution was monitored by absorption at 254 nm. Identification and quantitation of the amino residues were performed by comparison of retention times and peak areas with those of simultaneously and identically processed external standards. The standards were from the following sources: amino acid standards (Pierce, 20088); amino sugar standards D-glucosamine hydrochloride (Sigma, G-4875) and D-galactosamine hydrochloride (Fluka, 48250).

Results

New data on the amino sugar and amino acid compositions of the soluble fraction of modern and subfossil *Mya*

Table 1. Gradient conditions for reverse phase HPLC

Time (min)	Flow Rate (ml/min)	*Eluent A (%)	**Eluent B (%)
0.0	1.0	100	0
11.5	1.0	54	46
11.7	1.0	0	100
12.2	1.0	0	100
12.5	1.5	0	100
13.0	1.5	100	0
20.0	1.5	100	0
20.5	1.0	100	0

*Eluent A (75 mM sodium acetate, 0.1% triethylamine, 5% acetonitrile, pH 5.75).
** Eluent B (60% acetonitrile in dH_20).

truncata matrix, expressed as mean mole% of total amino residues detected, are presented in Table 2. The amount of soluble matrix recovered from the modern and subfossil shells was 706.35 pmol/µl and 2948.58 pmol/µl, respectively.

Data for the insoluble matrix from the same shells are presented by Risk et al. (in press) and are represented in histogram form in Figure 1, along with the data from the soluble matrix. These data were collected to complement a separate study of nuclear magnetic resonance analyses of protein/chitin ratios from *M. truncata* insoluble matrix.

Data were analyzed statistically for the two amino sugars and seventeen amino acids listed in Table 2. Values for soluble matrix compounds are given in Table 2 and Figure 1. Values for insoluble matrix compounds are tabularized in Risk et al. (in press) and also presented in Figure 1.

The modern and subfossil *Mya truncata* soluble/insoluble matrix samples each consisted of 35 randomly selected individual shells prepared in aggregate in order to provide sufficient material for HPLC analysis. Thus, amino sugar/amino acid compositions of each shell could not be determined. Analyses were conducted upon the following shell matrix fractions: modern soluble, subfossil soluble, modern insoluble, and subfossil insoluble. Three samples representing randomly drawn aliquots of the prepared matrix material were analyzed for the modern soluble, modern insoluble, and subfossil insoluble fractions; two samples were analyzed for the subfossil soluble fraction. Though statistical characterization of natural population variance within this experimental design was not possible, a statistical evaluation of analytical variation arising from the HPLC descriptions of these sets was accomplished. In addition, because the specimens were selected at random and involved a moderate number of individuals, it is expected that the findings are replicable at this level or in any larger experimental design which would allow for broader population inferences.

The mole% data were converted by arcsine transformation prior to statistical analysis. This transformation is recommended for use on percentage data (Sokol and Rohlf, 1969). Two datasets, one combining modern and subfossil soluble matrix data, the other combining modern and subfossil insoluble matrix data, were analyzed by a one-way ANOVA procedure for the comparison of amino compound group means. After determining that each one-way ANOVA proved significant with an F probability $\leq .00005$ and with a two-tailed Levene test for homogeneity of variance probability $\leq .0005$, post hoc methods were employed to examine the pairwise comparisons between modern and subfossil group means on each of the 19 amino compound groups. Because multiple comparisons were made within this context, a multiple range test known as Student-Newman-Keuls with a significance level of .05 was selected. This test provided for a moderate control of Type I error rate (false differences) by conducting all pairwise comparisons between means using the studentized range distribution. These

Table 2. Amino sugar/amino acid compositions of modern and subfossil soluble matrices of *Mya truncata*, expressed as mean mole%. Numbers in parentheses are the reconverted arcsine transformed 95% confidence intervals (Sokal and Rohlf, 1969). n = 3 (modern); n = 2 (subfossil). * indicates amino sugar; remaining compounds are amino acids.

Compound	Modern	Subfossil
glcn*	1.41 (0.66 - 2.39)	1.09 (1.05 - 1.14)
galn*	1.85 (1.46 - 2.28)	1.65 (1.53 - 1.77)
pro	19.1 (18.10 - 20.18)	16.7 (16.54 -16.88)
asp/asn	15.9 (15.10 - 16.68)	14.4 (13.82 -14.99)
thr	11.2 (10.70 -11.73)	7.08 (7.06 - 7.10)
ala	10.5 (9.96 - 11.07)	11.6 (10.68 -12.63)
gly	10.5 (10.18 - 10.79)	10.1 (10.05 -10.11)
ser	8.52 (8.44 - 8.60)	8.43 (8.24 - 8.62)
glu/gln	7.01 (6.66 - 7.38)	8.14 (7.30 - 9.02)
val	2.85 (2.68 - 3.03)	3.26 (3.11 - 3.42)
arg	2.45 (2.05 - 2.88)	2.83 (1.99 - 3.82)
tyr	2.21 (2.01 - 2.43)	3.69 (3.44 - 3.94)
leu	1.43 (1.17 - 1.71)	2.30 (2.19 - 2.41)
met	1.40 (1.07 - 1.76)	1.05 (0.94 - 1.15)
lys	1.31 (0.01 - 4.36)	3.62 (3.19 - 4.06)
ile	1.14 (0.93 - 1.37)	2.09 (1.98 - 2.21)
cys	1.08 (1.04 - 1.12)	1.00 (0.42 - 1.82)
phe	0.71 (0.68 - 0.75)	0.91 (0.32 - 1.79)
his	trace	trace

pairwise comparisons were conducted in a stepwise fashion where means were ordered from highest to lowest and the greatest differences were the first tested (Sokal and Rohlf, 1969).

The comparisons made with the soluble matrix data furnished significant differences between the modern and subfossil groups for 8 of 19 amino compounds. These are ASP/ASN, GLU/GLN, ILE, LEU, LYS, PRO, THR, and TYR.

The comparisons made with the insoluble matrix data furnished significant differences between the modern and subfossil groups for 10 of 19 amino compounds. These are ALA, ARG, ASP/ASN, CYS, GLU/GLN, GLY, PRO, SER, THR, and GLCN.

Discussion

The means of several modern and subfossil amino acid data pairs are significantly different for soluble and insoluble matrix residues. In addition, the amino sugar glucosamine is significantly less abundant in subfossil insoluble matrix samples than in modern insoluble matrix samples. The fact that readily measurable, significant differences occurred within a discrete time interval suggests that differences in relative abundance of matrix monomers between modern and subfossil *Mya truncata* shells may be useful for geochronological purposes. Bada et al. (1978), for example, studied the diagenesis of the amino acids serine and threonine in foraminiferal tests and derived empirical equations that related amino acid ratios to time values. These equations were useful for estimating the age of sediment samples containing foraminiferans. For *M. truncata* insoluble matrix samples, Risk et al. (in press) suggested that changes in the protein/chitin ratio as determined by NMR or amino acid/glucosamine ratios as determined by HPLC may be geochronologically useful, because chitin degraded more rapidly than protein within a measured time interval. Our new findings indicate that glucosamine and galactosamine compositions of collected soluble matrix material from *M. truncata* shells are not a promising source of geochronological information for the investigated time interval because of the lack of significant change in subfossil values compared to modern values. Numerous amino acids (and/or amino acid ratios), however, offer possibilities for further investigation (e.g. ASP/ASN, GLU/GLN, ILE, LEU, LYS, PRO, THR, and TYR in the soluble matrix). Nevertheless, the apparent increase in the absolute amount of soluble matrix in subfossil compared to modern samples is surprising (one would expect a decrease as the matrix is broken down during diagenesis) and likely complicates defining the true composition of the subfossil soluble matrix.

Risk et al. (in press) reported a 3.25-fold decrease in the amount of insoluble matrix recovered from subfossil shells compared to modern shells. The finding in this study of a 4.17-fold increase in the amount of soluble fraction residues in subfossil shells compared to modern shells therefore provides insights into the origin of the soluble residues obtained from *M. truncata*.

Both the insoluble and soluble matrices of invertebrates including mollusks are known to be affected by post-mortem hydrolysis of shell proteins (e.g. Goodfriend and Meyer, 1991; Goodfriend, 1992; Goodfriend et al., 1992) which result in racemization for particular monomers. For example, hydrolytic depolymerization may occur to the insoluble matrix, causing fragments to be solubilized. Hydrolysis of the soluble matrix is very rapid (<several hundred years) and lowers the molecular weight of soluble material. Considering the 8.7k BP age of the soluble matrix samples studied here, it is possible to envision at least two scenarios in which the composition of the collected soluble matrix may have been affected by post-mortem hydrolysis. First of all, hydrolysis of the original soluble matrix could have lowered the molecular weight of some of the soluble material below the 12-14 kDa level retained by the dialysis tubing used in sample collection, allowing this material to pass out of the tubing undetected. Secondly, hydrolysis of the insoluble matrix may have produced soluble material that was mobilized during the process of sample preparation and then retained by the dialysis tubing. Thus the "soluble matrix" recovered from the subfossil shells may differ in part from the soluble matrix of the modern shells as a result of the incorporation of a solubilized fraction from the insoluble matrix. The greater amount of soluble fraction recovered in the subfossil shells compared to the modern shells is evidence supporting this scenario. If this scenario is correct, then the "soluble matrix" collected for this study from the subfossil shells is an operational definition affected by diagenesis of other matrix components. It not only consists of original soluble matrix amino compounds but also likely contains solubilized fragments hydrolyzed from insoluble polymers.

We conclude that time-related changes in monomeric composition can be easily measured for both soluble and insoluble matrix residues of *Mya truncata*. We believe that these new findings should encourage further geochronological investigations of *M. truncata* organic matrix. Nevertheless, defining the true composition of the subfossil soluble matrix and inferring soluble matrix diagenesis is hampered because of contemporary degradation of the insoluble matrix.

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